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ELECTROPHYSIOLOGICAL AND OLFACTOMETER RESPONSES OF TWO HISTERID PREDATORS TO THREE PINE BARK BEETLE PHEROMONES

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Abstract—We measured electrophysiological responses in the antennae of two predaceous hister beetles, *Platysoma parallelum* and *Plegaderus transversus*, exposed to racemic mixtures of primary aggregation pheromones of scolytid bark beetle prey, ipsenol, ipsdienol, and frontalin. No significant differences were found for either histerid species between male and female antennal responses to any of the three pheromones. Measurement of antennal threshold responses indicated that *Pla. parallelum* has increasing antennal sensitivity to ipsdienol, ipsenol, and frontalin. In contrast, *Ple. transversus* exhibited similar detection thresholds to all three pheromones. *Pla. parallelum* antennae exhibited different response amplitudes to the three pheromones at quantities above the detection threshold, while *Ple. transversus* had similar responses to each. Behavioral responses to the same three pheromones were evaluated for both histerid species using pedestrian olfactometer bioassays. Both species were attracted to frontalin and ipsenol, but not ipsdienol. *Pla. parallelum* was significantly more attracted to frontalin than ipsenol, while *Ple. transversus* showed no significant preference for either compound. Our results suggest that histerids that prey upon pine bark beetles may have different host or host habitat preferences, which could reduce interspecific competition.

Key Words V Scolytidae, Histeridae, *Dendroctonus*, *Ips*, *Platysoma*, *Plegaderus*, bark beetle electrophysiology, hister beetle predators, host location, kairomone, attraction, frontalin, ipsenol, ipsdienol.

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INTRODUCTION

Hister beetles (Coleoptera: Histeridae) are important natural enemies of pine bark beetles (Coleoptera: Scolytidae). They comprise approximately 6Y7% of total predator abundance for southern pine beetle, *Dendroctonus frontalis* Zimmermann, and *Ips* spp. in the southern United States (Berisford, 1980; Kulhavy et al., 1989). Histerid adults and larvae feed primarily on the early life stages of bark beetles within their galleries, mined into the inner bark of pines (Kovarik and Caterino, 2000; WPS personal observations). They also facultatively prey upon secondary gallery fauna and are considered generalists within a specialized habitat (Erbilgin and Raffa, 2001). Histerids typically arrive at trees within 1 wk of bark beetle colonization (Shepherd and Goyer, 2003). The sympatric histerids, *Platysoma parallelum* (Say) and *Plegaderus transversus* (Say), are associated with both *D. frontalis* and *Ips* spp. infestations in the southeastern United States (Overgaard, 1968; Moser et al., 1971; Stein and Coster, 1977; Dixon and Payne, 1979; Goyer et al., 1980; Riley and Goyer, 1986; Shepherd and Goyer, 2003). These previous studies did not indicate any apparent host specificity for either histerid species.

Histerids exploit bark beetle aggregation pheromones as kairomonal attractants to locate host habitats (Dixon and Payne, 1980; Turnbow and Franklin, 1981; Payne, 1989; Shepherd and Goyer, 2003). Trapping studies have shown that histerids are attracted to the major aggregation pheromones of bark beetles of the southern United States, including frontalin (*D. frontalis*), ipsenol [*Ips grandicollis* (Eichhoff)], and ipsdienol [*Ips avulsus* (Eichhoff) and *Ips calligraphus* (Germar)] (Dixon and Payne, 1980; Turnbow and Franklin, 1981; Shepherd and Goyer, 2003). Variation in responsiveness to prey kairomonal odor cues may indicate that sympatric histerids use different strategies for host location. This could separate arrival times and locations on bark beetle-infested trees and thus reduce interspecific competition.

Our objective in this study was to determine differences in attraction to host pheromones between *Pla. parallelum* and *Ple. transversus* adults. We conducted both electrophysiological and behavioral assays with frontalin, ipsenol, and ipsdienol.

METHODS AND MATERIALS

Insects for Laboratory Assays. We collected adult *Pla. parallelum* and *Ple. transversus* predators from under the bark of loblolly pine, *Pinus taeda* L., logs naturally infested by *Ips* spp. at the Louisiana State University AgCenter, Idlewild Research Station, East Feliciana Parish, LA, USA. Histerids were maintained at room temperature (ca. 23°C) in glass petri dishes lined with moist

filter paper and were fed to satiation with *Ips* spp. larvae twice weekly. We used histerids in experiments up to 60 d after collection.

Electroantennogram (EAG) Recordings. For these analyses, EAG techniques were modified from those used by Visser (1979) and Scholz et al. (1998), and the equipment used was identical to that described in Asaro et al. (2004). We mounted intact head preparations of *Pla. parallelum* and *Ple. transversus* between two glass micropipette/gold electrodes filled with BeadleYEphrussi Ringer solution and 0.02% v/v Triton X-100 surfactant (Union Carbide, Midland, MI, USA), which improved electrical contact between the antenna tip and the electrode saline. The reference electrode was inserted into the base of the excised head, and the tip of the recording electrode was touched to the intact club of one antenna. The head preparation was enclosed within a brass Faraday cage.

We tested five dilutions (0.0001, 0.001, 0.01, 0.1, and 1 μg/μl) of synthetic racemic [50(+)/50(−)] ipsenol, 97% purity (Bedoukian Research, Inc., Danbury, CT, USA), ipsdienol, 95% purity (Borregaard, Sarpsborg, Norway), and frontalin, 97% purity (BASF, Ludwigshafen, Germany) in redistilled hexane. Dilution series of a single pheromone were delivered in random order to individuals of each sex and species: ipsenol (*Pla. parallelum* V 13 M/22 F, *Ple. transversus* V 18 M/10 F); ipsdienol (*Pla. parallelum* V 16 M/12 F, *Ple. transversus* V 18 M/8 F); and frontalin (*Pla. parallelum* V 12 M/17 F, *Ple. transversus* V 12 M/18 F). The number of replicates differed due to availability of vigorous histerids with undamaged antennae. In addition to the pheromone samples, we puffed a hexane-only control and standard solution (frontalin, *endo*-brevicomin, and verbenone at 0.1 μg/μl in hexane) before and after each puff of a sample dilution. We used a multiple-component standard mixture of *D. frontalis* pheromones because electrophysiological and behavioral responses by *Pla. parallelum* and *Ple. transversus* to individual compounds were not known. This standard elicited consistent, strong EAGs in both sexes and species in pilot trials.

Test solutions (10 μl) were applied to 10 × 0.5-cm strips of Whatman No. 1 filter paper inside glass Pasteur pipettes. We positioned the pipette tip 2 cm upwind of the head preparation in a continuous stream (400 ml/min) of humidified, charcoal-filtered air. Puffs of air (30 ml/min; 3-sec duration) were delivered from a Syntech (Hiversum, The Netherlands) CS-05 stimulus control unit. We recorded the peak voltage amplitude during the puff delivery of each stimulus as the antennal response. An interval of 1 min between puffs was found to be sufficient for complete antennal recovery in both species. We determined the sex of each beetle by dissecting the genitalia.

Y-Tube Olfactometer Bioassays. We tested short-range anemotaxic responses of *Pla. parallelum* and *Ple. transversus* adults in pedestrian bioassays, using a Y-tube olfactometer as described in Sullivan et al. (2000). Individual histerids were

introduced into the stem of a glass Y-tube (6-mm i.d., stem 7 cm, branches 7 cm, and 135° to the stem), and a choice was scored when a beetle walked 5 cm down one branch within 8 min of introduction to the Y-tube. Filtered, humidified air (30 ml/min, 23Y24°C, 50Y70% RH) carried odors from two bait-holding tubes to each branch of the Y-tube.

Baits were 100 μg racemic ipsenol, ipsdienol, and frontalin in hexane (10 μl) applied to filter paper squares (9 cm^2). The solvent was allowed to evaporate for 20 sec before papers were placed inside the sample tubes. Papers with ipsenol, ipsdienol, frontalin, and hexane only (control) were tested against each other in all possible binary combinations.

We used a total of 60 individual histerids of each species in each test and did not reuse them. Beetles were starved for 5 d prior to introduction to the olfactometer. Between trials, we replaced Y-tubes with clean ones and swapped bait treatments to opposite branches to eliminate directional bias.

Statistical Analysis. We calculated net EAGs by subtracting the mean responses to the controls introduced before and after the sample or standard from the actual sample and standard responses (Scholz et al., 1998). EAG data were standardized by calculating the percentages of the net EAGs relative to the standard solution (Payne, 1975; Dickens, 1978).

We used a Wilcoxon paired signed rank test to compare antennal responses to pheromone dilutions to the average of the contiguous control responses. Detection thresholds were calculated as the lowest concentration of pheromone producing significantly greater responses than the control. We compared histerid EAGs at and above the threshold for each species for sexes combined with a KruskalYWallis test and a Dunn's multiple comparison test (SAS Institute, 2001). A *G*-test for goodness of fit with William's correction for small samples was used to identify significant preferences for one olfactometer branch in the Y-tube tests (Sokal and Rohlf, 1995). We set significance levels at $\alpha = 0.05$ for all tests.

RESULTS

EAG Recordings. Mean net responses (±SE) of *Pla. parallelum* to the standard mixture were 2.01 ± 0.07 mV for males ($N = 41$) and 2.49 ± 0.05 mV for females ($N = 51$). For *Ple. transversus*, the mean net responses (±SE) to the standard were 5.45 ± 0.23 mV for males ($N = 48$), and 6.35 ± 0.21 mV for females ($N = 36$). No significant differences were found for either histerid species between male and female antennal responses to the three pheromones at any concentration. Thus, we combined male and female EAG data at and above the detection threshold for each species. *Pla. parallelum* and *Ple. transversus* both exhibited

TABLE 1. EAG DETECTION THRESHOLDS TO SERIAL DILUTIONS OF RACEMIC IPSENOL, IPSDIENOL, AND FRONTALIN FOR *Platysoma parallelum* AND *Plegaderus transversus* HISTERID BEETLES (SEXES COMBINED)

Species	Pheromone	Detection threshold (μg on filter paper)[a]	*P*-value
Pla. parallelum	Ipsenol	1	<0.001
Pla. parallelum	Ipsdienol	10	<0.001
Pla. parallelum	Frontalin	0.1	<0.001
Ple. transversus	Ipsenol	1	<0.001
Ple. transversus	Ipsdienol	1	<0.001
Ple. transversus	Frontalin	1	<0.001

[a]Lowest concentration of pheromone that elicited a significantly greater EAG than average of contiguous control responses ($P < 0.05$: Wilcoxon paired signed rank test).

significant antennal responses to racemic ipsenol, ipsdienol, and frontalin (Table 1). *Pla. parallelum* detection thresholds differed, with antennae exhibiting increasing sensitivity for ipsdienol, ipsenol, and frontalin (Table 1). In contrast, *Ple. transversus* detection thresholds were the same for all three pheromones (Table 1).

Mean percent EAGs for *Pla. parallelum* sexes combined were significantly higher for frontalin than ipsenol ($P < 0.001$) and ipsdienol ($P < 0.001$) and higher for ipsenol than ipsdienol ($P < 0.04$) at both 1- and 10-μg concentrations (Figure 1). For *Ple. transversus* sexes combined, there were no significant

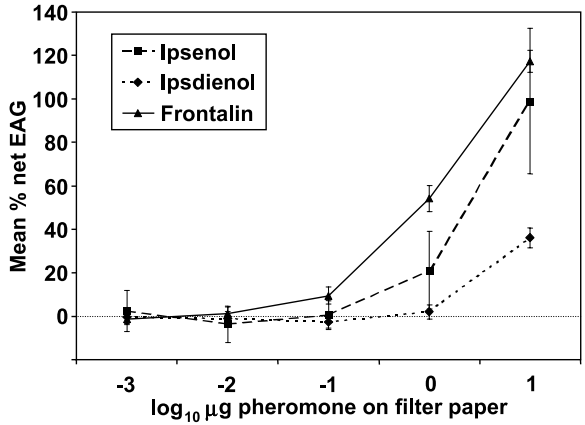
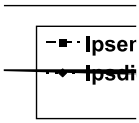


FIG. 1. Mean percent EAGs (±SE) from *Pla. parallelum* adults (sexes combined) to ipsenol ($N = 35$), ipsdienol ($N = 28$), and frontalin ($N = 29$), relative to the standard mixture of *D. frontalis* pheromones.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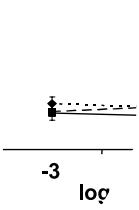
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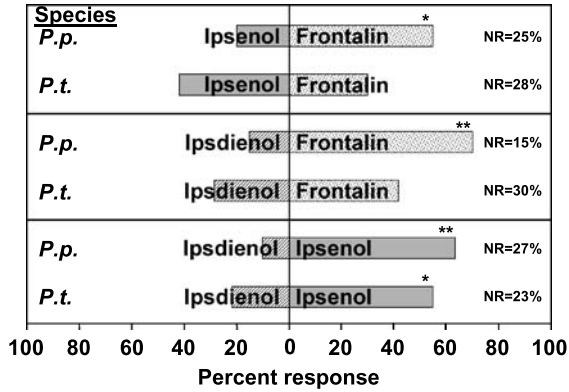

FIG. 4. Percentage of *Pla. parallelum* and *Ple. transversus* adults that walked toward either of two pheromone samples (100 μg each) in six paired choice tests using a Y-tube olfactometer. Asterisks indicate a significantly greater response toward one of the two choices using *G*-tests with William's correction for small samples (*$P < 0.01$; **$P < 0.001$). *P.p.* = *Pla. parallelum*. *P.t.* = *Ple. transversus*. NR = Percentage of histerids in each test that chose neither the pheromone sample nor the control within 8 min of introduction.

differences in responses to the three pheromones at or above the detection threshold (Figure 2).

Y-Tube Olfactometer Bioassays. Both *Pla. parallelum* and *Ple. transversus* were significantly more attracted to frontalin and ipsenol than the control (Figure 3). There was no significant attraction to ipsdienol for either species. Responses to the paired pheromone offerings differed for each species (Figure 4). *Pla. parallelum* was more strongly attracted to frontalin than ipsenol and ipsdienol and to ipsenol than ipsdienol. In contrast, *Ple. transversus* showed no significant preference for either frontalin vs. ipsenol or frontalin vs. ipsdienol when offered as paired choices, but preferred ipsenol over ipsdienol.

DISCUSSION

Different electrophysiological and behavioral responses to three primary bark beetle aggregation pheromones suggest that *Pla. parallelum* and *Ple. transversus* utilize different strategies for host or host habitat finding. The antennae of *Pla. parallelum* responded with increasing sensitivity to ipsdienol, ipsenol, and frontalin, indicating an ability to detect *D. frontalis* attack sites, from which frontalin odor plumes emanate, at greater distances than those of

Ips spp., and *I. grandicollis* colonizations at greater distances than those of *I. avulsus* and *I. calligraphu*s. This histerid likely has a larger antennal receptor population for frontalin than ipsenol and ipsdienol, as we recorded higher intensity antennal responses to frontalin for quantities above the detection threshold (Payne, 1975). We also recorded significantly higher intensity responses to ipsenol than ipsdienol for quantities above the detection threshold, providing evidence for a larger ipsenol receptor population (Payne, 1975). In contrast, *Ple. transversus* exhibited similar detection thresholds and EAGs above the detection threshold for all three kairomones and, thus, may have similarly sized receptor populations for these compounds. Electrophysiological studies of other bark beetle predators found that the clerids, *Thanasimus dubius* (F.) and *Thanasimus formicarius* (L.), also responded to multiple kairomones produced by different prey species (Hansen, 1983; Payne et al., 1984; Tommeras, 1985).

Since both histerid species were attracted to ipsenol and frontalin, but not ipsdienol in the olfactometer bioassays, they may preferentially orient toward portions of trees containing *D. frontalis* or *I. grandicollis*, rather than *I. avulsus* and *I. calligraphus*. The olfactometer preferences of *Pla. parallelum* were mirrored by greater electrophysiological responsiveness to frontalin and ipsenol. Attractive responses and the presence of a larger antennal receptor population suggest specialization for these kairomones. This histerid may be more attracted to trees or portions of trees containing *D. frontalis* than those with only *Ips* spp. In contrast, *Ple. transversus* had fewer odor preferences, differentiating only between ipsenol and ipsdienol when more than one kairomone was offered. Similar to its antennal responses, its attraction to prey kairomones appears less specific than those of *Pla. parallelum*. It may not distinguish between sites colonized by either *D. frontalis* or any of the *Ips* spp.

Differences in attraction patterns between these histerid predators may facilitate niche partitioning, reducing interspecific competition via spatial and temporal separation at sites infested with multiple bark beetle species. These histerids are generalist predators that have not been shown to associate preferentially with any of the four sympatric pine bark beetle species. Different kairomone response profiles may indicate previously unrecognized host preferences, and they may serve to stagger arrival times and separate landing sites at trees infested with multiple host species. In our study, both electrophysiological and behavioral data suggest that *Pla. parallelum* has a preference for frontalin over *Ips* spp. pheromones, while *Ple. transversus* exhibits little or no distinction among these compounds.

Complicating the interactions between these histerid species are the effects of various enantiomeric ratios of prey kairomones on behavior. Studies of histerids associated with *Ips pini* (Say) in Wisconsin have shown that *Platysoma cylindrica* (Paykull) was most attracted to traps baited with 25(+)/75(−)

ipsdienol (Raffa and Klepzig, 1989), and that *Pla. cylindrica* and *Pla. parallelum* were most attracted to traps baited with 3(+)/97(−) ipsdienol (Aukema et al., 2000a,b). In addition to bark beetle pheromones, histerids may use a variety of volatile odor cues derived from other potential prey, host trees, and microorganisms to locate their prey. Any of these compounds, individually or in combination, may provide optimum attractiveness to searching histerid predators.

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